

DNA Preparation from Adherent Cells

Section of Cancer Genomics, Genetics Branch, NCI
National Institutes of Health

Reagents

Chloroform

Mallinckrodt, cat. 4440

EDTA, 0.5 M

Ethanol, 100%

Ethanol, 70%

Isoamyl Alcohol

Sigma, cat. I-3643

Phenol

Invitrogen Corp., Cat. 15513-039

Phosphate Buffered Saline (PBS), 10X and 1X

Invitrogen Corp., Cat. 10010-023

Proteinase K

EM Science, Cat. 24568-2

Sodium acetate, 3 M, pH 5.2

Quality Biological Inc., Cat. 351-035-060

Sodium dodecyl sulfate (SDS), 10%

Tris-HCl, 1 M, pH 8.0

TAE buffer (Tris acetate/disodium EDTA), 1X

Bio Whittaker, Cat. 16-011V

Trypsin

Invitrogen Corp., Cat. 25200-056

Distilled Water

Invitrogen Corp., Cat. 15230-170

Preparation

DNA buffer

1M Tris-HCl, 1 M, pH 8.0	100 ml
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0.5 M EDTA	100 ml
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dH ₂ O water	300 ml
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Chloroform/Isoamyl alcohol 24:1

Chloroform	24 ml
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Isoamyl alcohol	1 ml
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Procedure

1. Use trypsin or cell scraper to remove cells from tissue culture flask (T-75). Centrifuge cultured cells for 10 min at 10°C (1,200 rpm). Remove supernatant and re-suspend cell pellet in 1X PBS and wash twice with 10 ml 1X PBS, centrifuging between washes.
2. Resuspend pellet in 10 ml DNA buffer. Centrifuge cells for 10 min at 10 °C (1,200 rpm). Remove supernatant.
3. Add 3 ml DNA-buffer, re-suspend the pellet, add 125 µl Proteinase K (10 mg/ml) and 400 µl 10% SDS; shake gently and incubate overnight at 45°C.
4. Add 3.6 ml of phenol, shake by hand for 10 minutes (RT); centrifuge for 10 min at 10°C (3000 rpm).
5. Transfer the supernatant into a new tube (15 ml); measure the volume. Add 1.8 ml phenol and 1.8 ml chloroform/isoamylalcohol (24:1) or a total amount equal to the volume of the supernatant. Shake by hand for 10 min (RT); centrifuge for 10 min at 10°C (3,000 rpm).
6. Transfer the supernatant into a new tube (15 ml); measure the volume. Add 3.6 ml chloroform/isoamylalcohol (24:1) or an amount equal to the volume of the supernatant. Shake by hand for 10 min (RT); centrifuge for 10 min at 10°C (3,000 rpm).
7. Transfer the supernatant into new tube, measure the volume. Add 1/10 volume 3 M sodium acetate (pH 5.2) and 3 x the volume 100% ethanol; shake gently until the DNA is precipitated.
8. Use a sterile glass pipette to transfer the precipitated DNA into a tube with 30 ml of 70% ethanol tube. Place on inverting rack and invert for 2 hr to thoroughly rinse. Transfer DNA into a sterile eppendorf tube.
9. Centrifuge for 20 min at 14,000 rpm. Dry pellet in a SpeedVac for 5 min. Dissolve the DNA in 300-500 µl sterile water and place in an eppendorf thermomixer shaker overnight at 37°C.
10. Measure the DNA concentration and run 1-5 µl (approximately 200 ng) for gel electrophoresis on agarose gel (1%) in 1X TAE buffer. Also, measure the DNA with NanoDrop and print out the results for future reference.